Chapter 7

# Pepper Crop under Climate Change: Grafting as an Environmental Friendly Strategy

Consuelo Penella and Angeles Calatayud

Additional information is available at the end of the chapter

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**Abstract**

Pepper is an extremely important vegetable worldwide in socio-economic terms. However, persistent land use, monoculture, and intensified production processes have led to soil diseases. This, along with abiotic stress, and mainly salinity of soil and waters, water stress, and suboptimal temperatures, can lead to physiological disorders emerging in peppers, e.g., cracking and Blossom end rot, which induce plant senescence, and lower not only in yields, but also in product quality. Salinity and water shortage are the two main environmental problems that crops face in the Mediterranean Region. One way of overcoming stresses from an ecological or integrated crop management viewpoint is to use grafted plants as an adaptation strategy. Initially, grafting technology has expanded in Solanaceae and Cucurbitacea species to overcome biotic stress. Nowadays, grafts are being used as several approaches to cushion the impact of climate change on agricultural systems. Furthermore, grafts allow desirable varieties by organoleptic or productivity traits, but they are sensitive to abiotic stress and can be grown under abiotic stress. As far as we know, very few studies on grafted pepper plants under abiotic stress are available.

**Keywords:** abiotic tolerance, drought, graft, pepper, salinity

## 1. Introduction

Peppers, chiles, capsicum, or no matter what other name they come under, are versatile crops included in most daily diets, especially in some areas more than others. Capsicum plants are topics crops that better grow in hotter zones [1]. They are eaten fresh, dehydrated and processed, and also as a spice. Given its vast versatility, peppers are being increasingly eaten, but also due to the fact that they are a major source of pro-vitamin A (carotene), E ($\alpha$-tocopherol), and one of its main attributes is vitamin C (ascorbic acid). Mature pepper fruits are rich in

carotenoids, compounds with anti-carcinogenic and antioxidant ability. Mature and immature fruits contain high contents of phenolics, especially flavonoids for which there are reports of antioxidant and other bioactive properties [2–4], and plenty of essential nutrients.

According to their culinary purposes and organoleptic features, pepper fruits are normally classified as two kinds. One is a bell pepper, which means a non-pungent, chunky sweet pepper kind, whereas chilli pepper refers to pungent chilli fruits [1]. Generally speaking, non-pungent peppers are more popular in the northern hemisphere, but more pungent chilli peppers are eaten more in tropic and subtropic areas [5].

Peppers grow in most countries on our planet, and they cover 1.93 million ha of crop-growing surface area. As a spice and vegetables, the world's pepper production has gone from over 12 million tons in 1993 to more than 31 million in 2013 over the past 20 years [6]. China is the largest pepper producer (almost 16 million tons) and is followed by Mexico (2.3 million), Turkey (2.2 million), and Indonesia (1.8 million) (**Figure 1**).

Peppers have adapted well to hot climates. The optimum seed germination temperature is 25–30°C. For fruit quality and growth purposes, areas with temperatures within the 21–29°C range are needed [7]. When temperature goes under 15°C or exceeds 32°C, growth can be retarded, and blossom end rot (BER), fruit-set ceases may emerge, with lower yields [8]. Generally speaking, commercial pepper varieties need friable, well-drained, sandy loam soil with pH of 6.5–7.5 for optimum production. Salt content in soil and irrigation water should be low. There are reports of a salinity resistance threshold of 1.5 dS $m^{-1}$, below which no effect on growth occurs, and a 14% drop in biomass production per additional 1 dS $m^{-1}$ has been found [9]. Thresholds ranging from 0 to 2 dS $m^{-1}$, and slopes of salinity response curves that go from 8 to 15%, have been indicated for greenhouse peppers [10, 11]. Added organic matter increases the water-holding capacity and supplies minerals and nutrients. Peppers need high frequent soil fertility at the start of the growing cycle to supplement N. If water is lacking or excessive, flower abortion or further BER of fruits can be induced [12].

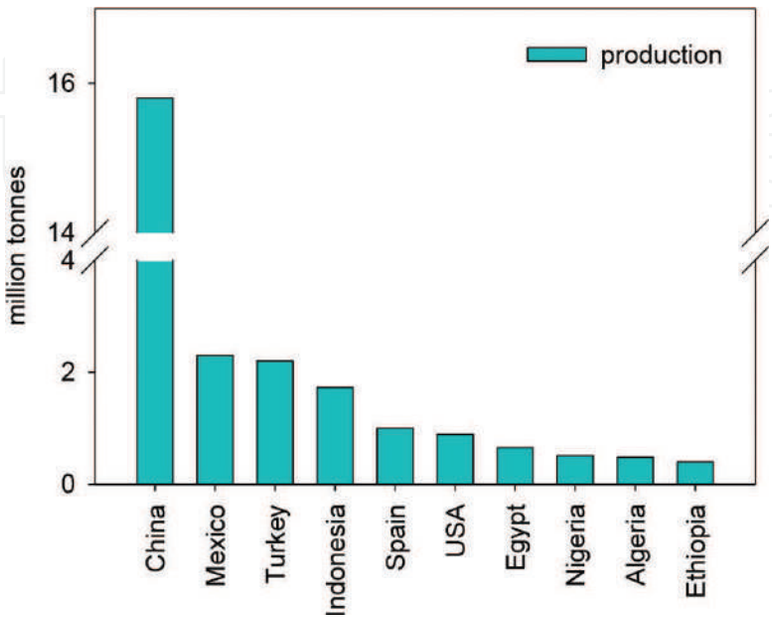


**Figure 1.** World production of chillies and peppers by country (million tons) [6].

### 1.1. Historical and botanical perspectives

The Solanaceae family is a complex that comprises at least 98 genera and as many as 2716 species, including Capsicum [13, 14]. This family also includes other major crop types, like potato, eggplant, tomato, and tobacco. "Capsicum" comes from a Greek-based derivate of the Latin "Kapto," which means "to bite," and refers to heat (pungency). Capsaicin, which is a volatile molecule, is also a very stable molecule that is responsible for the pungency normally linked with certain peppers [15]. Other pepper species are non-pungent because of a single mutation, which leads to the inability to generate capsaicinoids.

The genus Capsicum has been found in the central hemisphere and in South America ever since civilization began. It is likely to have evolved from an ancestral form in Bolivia-Peru. It formed part of human diet at approximately 7500 BC [16]. Peppers were completely unknown in Europe, Africa, and Asia before Columbus landed in the Americas. During his voyage, he came across a plant with fruit that resembled the pungency of black pepper, *Piper nigrum* L. The genus Capsicum, more commonly known as "pepper," "capsicum," "red chile," "bell pepper," "chilli pepper," "paprika," "tabasco," "cayenne," etc., contains up to around 40 species. The vast phenotypic variation comprises fruit shapes, colors, sizes, and plant habits [17]. Capsicum species, with barely any exceptions, are diploid (2n = 24, less frequently 2n = 26) with similar karyotypes [18, 19]. Wild and cultivated chillies possess morphological differences that can be easily discerned. The fruit of all wild chilli forms come as small, berry-like red fruits, and birds are attracted by their sizes and colors. *Capsicum annuum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L., *Capsicum baccatum* L. (C. var. pendulum), and *Capsicum pubescens* R & P are the five main cultivated or half-cultivated Capsicum species [1, 20, 21]. *C. chinense*, *C. annuum*, and *C. frutescens* form a closely linked group, also known as "annuum Complex" [22] which, according to some authors, are not differentiated species.

## 2. Main environmental problems to cultivate pepper plants

In the face of climate change, global food security demands increasing agricultural production on finite arable land that does not increase water use [23]. As the world's population is estimated to increase to about 9 billion by 2050, the World Food Summit on Food Security (2009) has set a target of a 70% global food production increase. Environmental stresses are the most limiting conditions for plant exploitation and horticultural productivity worldwide [24, 25]. The most limiting factors include temperature, water availability, light, salinity, pathogens, and metal ion concentrations. Many disorders and diseases can interfere with pepper production and its quality, which can be of biotic (living) and abiotic (non-living) origin.

### 2.1. Biotic stresses

Capsicum plants can be attacked by distinct pathogens. The most troublesome and important pests and diseases are: fungal diseases, like *Phytophthora capsici* (**Figure 2A** and **B**), *Rhizoctonia solani*, *Verticillium dahliae*, Fusarium spp., bacteria, e.g., *Xanthomonas campestris*, and powdery mildew (*Oidiopsis taurica* and *Leveillula taurica*), viruses (**Figure 2C**), like Tomato Spotted Wilt Virus (TSWV), Pepper Mottle Virus (PMV), Beet Curly Top Virus (BCTV), several Mosaic Virus

(AMV), (CMV), (TMV), nematodes, chiefly *Meloidogyne incognita*, and insects (**Figure 2D–I**), e.g., mites, termites, aphids, and thrips.

Biotic stresses can bring about physiological changes in pepper plants, e.g., ion-flux change, electrolyte leakage, activation of defensive responses, and hypersensitive cell death [26]. These effects can result in smaller yields and worse quality. One of the most hazardous biotic factors is soil diseases, especially for intensive farming, where soil-borne pathogens can build up if crop rotations are limited. The main injuries to roots of these soil pathogens include smaller foliar size, thin weak stems, wilting, depressed flowering, worse fruit quality, and shorter plant life spans [27]. Initial symptoms are quite visible on leaves when plant roots have been completely infected. Farmer's only feasible option is taking preventive measures, which involve soil treatments for the next crop season. As soil fumigation with methyl bromide (MB) is forbidden, other alternatives need to be taken [28]. Fumigants are an option, but vast amounts can be applied that might result in phytotoxicity [29, 30]. Furthermore, the long-term use of fumigants

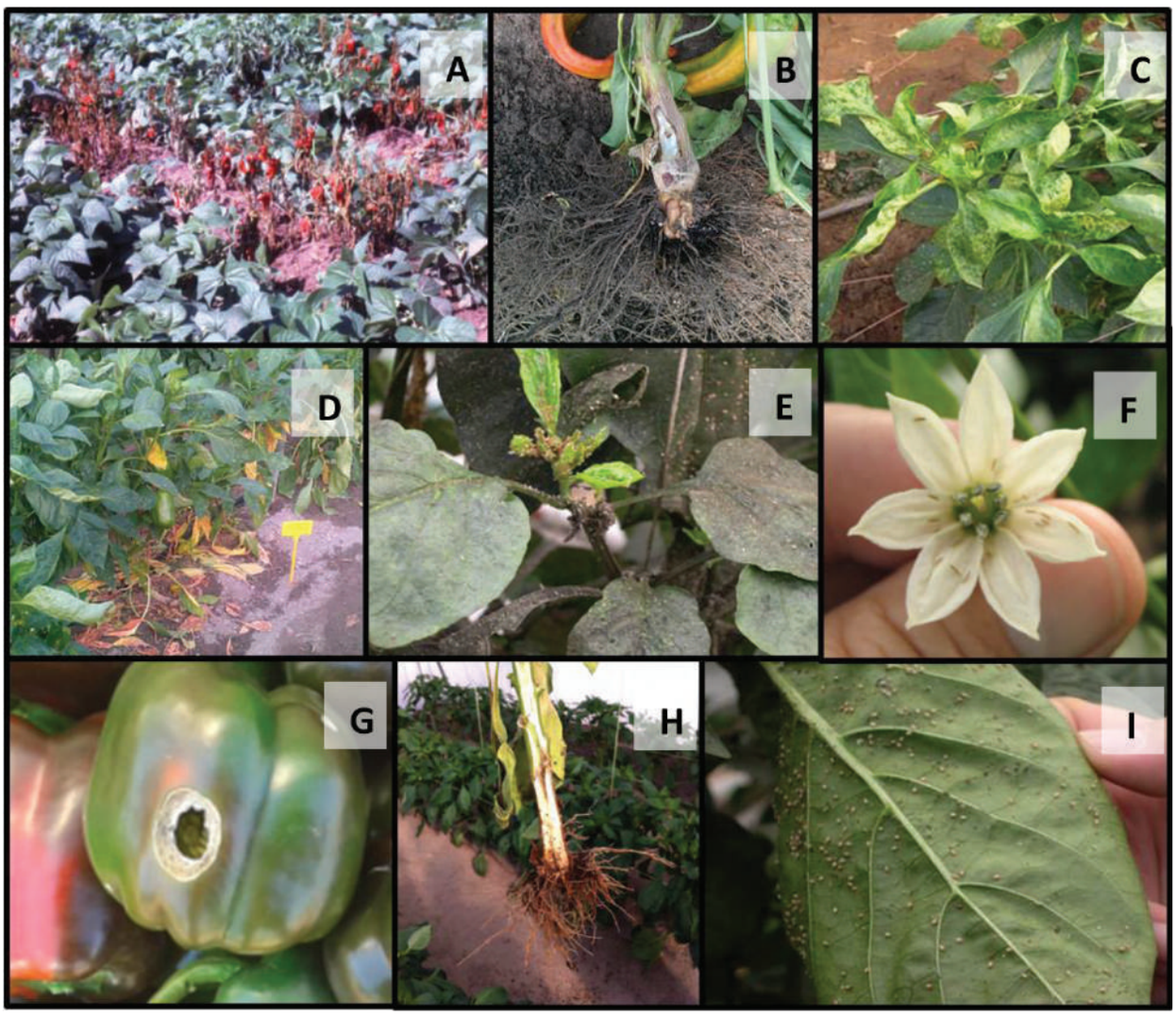


**Figure 2.** (A) overview of a pepper field infected by *Phytophthora capsici* (courtesy of Juan José Tuset), (B) detail of roots infested with *P. capsici*, (C) virus (courtesy of J.I. Marsal), (D) overview of a pepper field with mites; leaf discoloring and defoliation, (E) buds parasitized by aphids (courtesy of A. Miguel), (F) flower detail with thrips (courtesy of J.I. Marsal), (G) pepper bitten by an insect, (H) stem affected by termites, and (I) details of aphids on a leaf (courtesy of J.I. Marsal).

may lead to changes in the microfauna of soil, which not always favors cultivated plants [31]. Steam treatment is not toxic and effectively kills pathogens, but is not an economically feasible option everywhere as it requires suitable steaming machinery, and also fuel and water [32]. Soil solarization is used frequently in countries with a warm climate [33], but soil must be covered for 4–6 weeks with hot periods to stop vegetable production. Another alternative is biological control, which involves selecting organisms based on their ability to control diseases, which can be used for aerial plagues.

Another possibility is plant biotic resistance. To enhance crop tolerance, many attempts have been made by traditional breeding programs. Although commercial success is limited given trait complexity, commercial cultivars with some tolerance are found. In the present-day, vast efforts have been made to genetically transform plants to improve their tolerance. Although some increased tolerance to pathogens has been reported in transgenic peppers [34, 35] other approaches to achieve resistance must be currently considered as genetic engineering means in plants has been poorly accepted by the public [36].

One way to reduce or avoid lost production is to graft sensitive plants onto robust rootstocks. Several *Capsicum* rootstocks, including breed lines, commercial cultivars, and wild accessions, can contribute adequate tolerance or resistance to *Phytophthora*, *Fusarium*, *Verticillium*, CMV, nematodes, etc. [37–39].

### 2.2. Abiotic stresses

During the growth cycle of peppers, as with other plants, many unfavorable environmental conditions can occur, such as salinity, drought, extreme temperatures, moisture, light, mineral deficiencies or toxicities, pH, and pollutants, which can all diminish plant yields [21, 40, 41]. Close to 82% of the potential crop yields is lost yearly from abiotic stress, and the quantity of available productive arable lands continues to drop worldwide, which forces farmers and farms to move to places with a higher abiotic stress potential [42].

In the Mediterranean Region, one of the most important abiotic stresses is salinity, which is usually present in both soil and water, as well water scarcity, but improving these environmental conditions through crop management is very difficult.

Some other abiotic stresses include: low temperature because it affects pepper vegetative development and reproduction as it disturbs how flower female organs function, and the amount of viable pollen grains per flower [43, 44]; high temperature and radiation promote stunted growth, a lower photosynthetic rate, increased respiration, and poor water and ion uptake [24, 26]. Therefore, using different shading screens is considered an alternative to overcome these problems [45, 46]. Likewise, heating is used to avoid chilling and frost injury, and cooling is employed to avoid high air temperatures [21].

#### *2.2.1. Drought stress*

Water scarcity is believed to be a major threat for the twenty-first century (UNESCO, 2012). During their life cycles, plants are subjected to periods of soil and atmospheric water deficit. Indeed, about only 15% of agricultural land is irrigated worldwide, but irrigated lands make up nearly 50% of the world's food production [47]. Drought, along with salinity, is one of the most important

causes of low yields worldwide [48]. Adapted cultivars can improve the synchronization between crop water demand and soil supply. For all these reasons, we need to know plant responses to water scarcity, which are complex, and involve deleterious and/or adaptive changes [49].

As soil dries, its matric potential becomes more negative [50]. Plants can continue to absorb water only as long as their water potential (Ψw) is lower (more negative) than that of soil. The water potential is the total of both the solute potential (Ψs) and the turgor potential (Ψp): thus: $\Psi w = \Psi s + \Psi p$ [51]. In this way, one of the important pathways to enhance water stress tolerance is through osmotic adjustment, which maintains the leaf turgor required for stomatal opening, and to hence sustain photosynthesis and growth [52, 53]. Plants accumulate various types of compatible solutes, such as sugars, proline, glycinebetaine, or potassium [53, 54] to lower the osmotic potential and to absorb water. Basically, cells' accumulation of solutes is a process by which the water potential can lower without being linked to an accompanying reduction in turgor or a reduced cell volume.

Stomatal closure and reduced transpiration rates are prompt responses under drought stress because they lower the water potential of plant tissues. As a result, photosynthesis lowers, mediated by diminished $CO_2$ availability that is caused by: (a) diffusion limitations via the mesophyll and/or stomata [55], known as stomatal effects; (b) altered $CO_2$ fixation reactions, mediated by reduced Rubisco activity, known as non-stomatal [56]. With water stress, as energy accumulates in plants, which consume less light energy through photosynthetic carbon fixation, reactive oxygen species (ROS) generation increases [57, 58]. Accumulation of sorbitol, mannitol, and proline, and the formation of radical scavenging compounds, e.g., ascorbate, glutathione, and $\alpha$-tocopherol, can help plants to cope with water stress [59]. Such compounds play a dual role as the non-enzymatic antioxidants needed by plants to counteract the inhibitory metabolic effects of the ROS generated under water stress [60], and also in stabilizing proteins and enzymes, and in protecting membrane integrity [61]. Besides these physiological responses, plants also undergo morphological changes [62], like stunted growth and, consequently, smaller yields.

Generally, pepper plants are sensitive to water deficit due to big leaf areas and higher stomata conductance [63–65]. In the pepper production industry, drought imposes huge reductions in crop yields and quality, with significant economic losses of up to 70% [64, 66, 67]. The two most critical moisture stress stages in peppers are the initial establishment of transplanted plants and the stage prior to blossoming [17]. Thus, reduced yields and smaller fruits are frequently recorded under moisture stress conditions. Moreover, this scenario limits the water applied to peppers during rapid growth periods to reduce final yields [68].

*2.2.2. Salinity*

Salinity can be disastrous because it can have many direct and indirect harmful effects. It inhibits seed germination, induces physiological dysfunctions and often kills non halophyte plants, even at low concentrations, and also limits agricultural development [69, 70]. Salinization transforms fertile and productive land into barren land, and often leads to habitat and biodiversity loss [71]. Salt accumulating in excessive amounts in cultivated soils is a common problem, especially under irrigated conditions, which threatens food production globally [72, 73]. The indiscriminate use of large quantities of chemical fertilizers and overexploitation of

aquifers have dramatically multiplied the surface area affected by salinity [27]. Today to a greater or lesser extent, a third of all irrigated lands worldwide is affected by salinity [74], which means smaller yields.

Salt stress has two components that negatively affect plant growth: osmotic component and ionic component. A high salt concentration lowers the water potential in soil, and results in water stress in plants, known as the osmotic salinity component. The accumulation of given toxic ions represents the ionic component [75].

The relative degree of each salt effect caused by different salinity levels and its consequences on crop production are not clearly understood [67]. Saline soils induced by protected culture are complex and can include high concentrations of $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$, $NO_3^-$, and $Cl^-$, which differ from the saline soils induced by seawater, in which NaCl is the most soluble and widespread salt [52, 76]. High $Na^+$ concentrations lower $Ca^{2+}$ and $K^+$ uptakes, which leads to reduced stomatal conductance that results in lower $CO_2$ concentrations and, consequently, lower photosynthesis. High $Cl^-$ concentrations cause chlorophyll degradation and reduce actual quantum yields of PSII electron transport [77].

Salinity causes membrane destabilization [78], nutrient imbalances [79] and irreversible harm to plant tissues and cells [80]. It is well-accepted that growth inhibition by salt stress is linked with alterations to the hydric relationships in plants as a result of osmotic effects with certain ionic consequences.

Salt tolerance mechanisms include: (i) salt exclusion: plants limit salt accumulation in tissues by inhibiting root uptake. Some salt transport restriction strategies to sensitive tissues or organs have also evolved [81]. Plants' ability to regulate the transport and uptake of salts depends on these mechanisms: root cells' selectivity of uptake; preferential loading of $K^+$ instead of $Na^+$ onto the xylem by stele cells; salts removed from the xylem in upper root parts, leaf sheaths, and the stem according to the exchange of both $K^+$ and $Na^+$; (ii) salt excretion: halophytes often have anatomical structures, like salt bladders and salt glands, that are designed to eliminate any excess salt ions from plants to their environment; and (iii) intracellular ion compartmentation. The sequestration of ions or salts into leaf and/or shoot vacuoles is typically attributed to dicotyledonous halophytes. Such accumulation depends on tonoplast $Na^+/H^+$ antiporters and vacuolar $H^+$-translocating transporters that are induced by saline environments [82]. One immediate salt stress effect is cell alkalinization, which is linked with the $Na^+/H^+$ antiporters activity of tonoplast vesicles [78]. Here different types of compatible organic solutes and potassium ions, like proline and soluble sugar, accumulate in the cytoplasm to avoid dehydration and to maintain the osmotic-ionic balance between both two compartments [83], and to also stabilize subcellular structures, e.g., proteins and membranes [52, 84]. It has been observed in tolerant salt plants after the initial loss of cellular turgor that plants are able to induce an osmotic adjustment to the lower external water potential by compartmentalizing toxic ions in the vacuole and then synthesizing compatible solutes in the cytoplasm [78].

Pepper, and *C. annuum* in particular, is highly susceptible to salt stress. Negative effects on yields stem from disturbances the following: membrane permeability, water channel activity, ion imbalance, poor total photosynthesis, and stomatal conductance, which modify the carbon balance required to maintain both productivity and growth [72, 85–87].

## 3. Main disorders related to abiotic stress in pepper plants

### 3.1. Blossom end rot

Blossom end rot (BER) is a serious disorder known to affect peppers that grow under different environmental stresses. BER symptoms are linked with membrane leakage of cell solutes, cell plasmolysis, and membrane breakdown [88–90]. Thus fruit surfaces display water-soaked symptoms, and the tissue at the distal fruit portion ends up becoming discolored and necrotic. BER causes premature ripening and enhances fruit softening, which result in small-sized fruits [91] (**Figure 3**). In internal fruit tissues, BER develops in the necrotic region of the parenchymal tissue surrounding young seeds, and also in the distal placenta [89]. It is predominantly viewed that the cause of BER is inadequate calcium translocation to the fruit tip for rapid fruit expansion, which takes place under conditions that favor rapid fruit growth, e.g., bright light and high temperature. Hence, cell integrity is impaired with consequent tissue disintegration [92]. Since $Ca^{2+}$ is thought to play a key role, BER is termed a "calcium-related disorder" [93]. BER incidence is related to environmental factors, like high salinity, water scarcity, high temperature, and ammonia nutrition, which contribute to $Ca^{2+}$ deficiency [91, 94, 95]. However, a close relationship between calcium levels and BER cannot always be demonstrated [90]. Lantos [96] has shown that applying calcium does not necessarily reduce the yield losses caused by calcium deficiency.

The influence of stress on BER which occurs in peppers is partly based on not only increased NAD(P)H oxidase (an oxygen radicals-generating enzyme) activity, but also on higher ROS production, e.g., superoxide radicals, hydroxyl radicals, and singlet oxygen ($O_2$) in fruit apoplasts [91, 92, 97]. ROS are known to trigger cell death, which is characterized by the progressive loss of membrane integrity to result in cytoplasm swelling, and also in the release of cellular constituents [98], including loss of $Ca^{2+}$ ions, which may explain the lower $Ca^{2+}$ concentrations found mainly in the apoplast [88]. A certain amount of stress, caused by either a single or an interaction of several environmental factors, like high relative humidity, pathogenic stem diseases, and dry or saline soils, may have a negative effect on calcium uptake [99], which does not always end in a corresponding degree of BER [90].

**Figure 3.** Overview of the pepper fruits affected by BER (right) and details of necrotic tissue (left).

Two phytohormone types appear to especially interfere with BER affection, and also in opposite directions: abscisic acid (ABA) and bioactive gibberellins (GAs). The antagonism action between vegetative growth and $Ca^{2+}$ has been reported by Lyon et al. [100]. Low $Ca^{2+}$ in the nutrient medium has been indicated to result in very extensive root systems, which suggests great GA activity. Accordingly, a low $Ca^{2+}$ supply might have caused the high BER incidence more indirectly through enhanced GA activity [88]. ABA, as an antagonist to GAs, is known for reducing plant susceptibility to stress; e.g., by promoting $Ca^{2+}$ transport to fruits. Applying ABA to highly stressed tomato plants has been recently demonstrated to alleviate BER symptoms [101].

From a practical point of view, GA-signaling can be reduced by, for example, root restriction [102], by applying growth-retarding chemicals, and also by ABA [103, 104].

Basically, BER development involves several steps: stress enhances ROS production; ROS leads to lipid peroxidation with greater membrane leakiness which, in turn, leads to the rapid vacuolation of parenchyma cells and to loss of ions, which includes water-soluble apoplastic $Ca^{2+}$. This situation is also aggravated when plants are grown vigorously, when GAs levels are high and when ABA is low. All these are typical BER symptoms [94]. Thus final $Ca^{2+}$ deficiency can be considered a result, but not the cause, of only BER.

To control BER solutions, reducing susceptibility to stress and alleviating stress severity are necessary by: (i) proper selection of suited production sites. However, this is not always possible, and environmental conditions are unpredictable; (ii) improving management practices, e.g., shade or applying calcium fruit sprays. However, not enough evidence is available to recommend their use to manage BER; or spraying ABA, which remains unavailable as a commercial solution (no commercial formulation and side effects); (iii) breeding and selecting stress-resistant cultivars. Sadly, programs are slow and obtaining a variety that collects commercial fruit attributes and a robust radicular system is difficult; (iv) robust rootstocks inducing higher production in horticultural crops, which leads to a larger leaf area in grafted tomato plants [105], maintains a greater net $CO_2$ assimilation in grafted cucumber plants [106, 107], and has also shown a vigorous root system that increases the absorption of water and minerals in pepper-grafted plants [108]. Thus, grafting susceptible plants onto robust rootstocks to reduce their susceptibility to stress can reduce the fruits affected by BER, maintain water uptake, contribute to better plant nutrition; consequently, calcium deficiency can diminish [109–111].

### 3.2. Fruit cracking

Fruit cracking is yet another frequent physiological disorder that lowers marketable fruit yields, but it is not such a serious commercial problem as BER. The cracks in cracked fruits normally spread through the wall into the locule area because of repeated shrinkage. Such expansion weakens fruit cuticles [112]. Incidence is affected by environmental factors, mainly by varietal characteristics [113]. Several studies have demonstrated the importance of the environment in cuticle cracking development, like low night vapor pressure deficit [114], relative humidity [115], and temperature [116]. Fruits that display a wider expansion-shrinkage amplitude are often associated with severe cracking symptoms. The water status of fruits is a key factor to establish fruit cracking severity [21]. Some solutions can include those that minimize changes in their water status. Indeed, the same strategies used to combat BER can be adopted. Nonetheless, maintaining a consistent optimized growing environment is the best way to avoid fruit cracking.

## 4. Coping with abiotic constraints

The impact of both unpredicted climate change and climate variability on agricultural productivity is most likely to become a major constraint to achieve greater food production, which means that developing crop genotypes that withstand ambient stresses a major food security strategy. Hence, crop improvement innovations are needed [117]. They entail making furious efforts, especially by breeding companies that use conventional breeding programs. However, commercial success is extremely limited given the complex trait and practical selection tools are lacking; e.g., genetic markers have rendered these tasks inefficient and slow processes to date [84, 118, 119]. Combining suitable commercial fruit characteristics (quality and high production) and resistance to environmental factors is extremely difficult, especially when growing traditional varieties for their adaptation and traits quality since they are highly stress-sensitive [120, 121].

More recently, major efforts have been made to achieve genetic transformation [122–124]. Transferring a single gene or a few genes has led to claims of improved abiotic stress tolerance [125, 126]. However, the nature of genetically complex mechanisms of abiotic stress tolerance, and any potential detrimental side effects, makes this task most difficult [118, 127]. Lack of public acceptance of genetic engineering means that searching for other strategies to generate improved tolerances to abiotic stresses in plants is a priority [63, 128].

One environmental-friendly technique for avoiding or reducing loss in commercial yields caused by abiotic stress conditions is to graft susceptible commercial cultivars onto rootstocks that are capable of reducing the negative effect of external stress on shoots [25, 27, 129, 131]. Using grafted plants is an eco-friendly strategy that allows plants to overcome both soil-borne diseases and environmental stress [25, 110, 132].

### 4.1. Grafting

Grafting is defined as the natural or deliberate fusion of plant parts to establish vascular continuity among them [133], as well as the resulting genetically composite organism functions as a single plant [134]. The term scion denotes the shoot piece that stems from a donor plant that will be the grafted plant's canopy. The term rootstock indicates a plant that receives and fuses with the scion, and functions as the grafted plant's root system.

Despite vegetable grafting being an ancient practice, grafting did not become a common practice in ornamental and herbaceous vegetables before the twentieth century [135]. Cultivating grafted horticultural plants began in Korea and Japan toward the end of 1920s by grafting watermelon plants to squash rootstocks [136]. Ever since, this technique has been employed in watermelon, melon, cucumber, eggplant, pepper, tomato, and ornamental cactus and has exponentially increased. Grafting is also utilized for untypical fruit vegetables like artichoke [137, 138]. The advantages that vegetable grafting offers are attributed mainly to rootstocks' resistance to soil-borne diseases (fungus, nematodes, and bacterial wilt), and also to better vigor and stress tolerance. The problems related with banning methylbromide for soil fumigation purposes have led to increased vegetable grafting in the USA and Europe in recent years.

Micro- and tube-grafting and cleft approach are techniques that reliably combine pepper scions with compatible rootstocks, and the same can be stated of tomato and eggplant [139]. Recently, tube-grafting has become the most popular method type. It consists in cutting the growing rootstock tip at an angle of 45° below cotyledons and attaching it to the scion, which has been preciously cut at the same 45° angle above cotyledons, and then using a clip to fix the rootstock and scion (**Figure 4**).

Commercial varieties are not normally chosen to cope with abiotic stress. So, an interesting method to cope with these problems is to graft onto robust rootstocks.

Although grafting is a widespread eco-friendly technique applied in melon, tomato, or eggplant, it has been exploited less in peppers. This is basically because rootstock genotypes are lacking, which are simultaneously tolerant to biotic or abiotic stresses and can also improve commercial yields to amortize the extra costs incurred by grafting.

The main reason for grafting pepper is to improve plant vigor, disease tolerance, and uniformity, but very few commercial pepper rootstocks are available. This is because attention has been paid mainly to biotic stresses, and only the high-value pepper transplants utilized for protected cultivations are produced as grafted plants [39, 140, 141].

However, the abiotic stress incidence is very high, and increasing global climate change is forecast, while salinity and water stress are found frequently in areas where peppers are growing. It is necessary to perform several screenings to find Capsicum plants that tolerate abiotic stress so they can be used as rootstocks. In order to select the appropriate rootstocks,

**Figure 4.** Pepper seedling grafted by the tube-grating method.

searching for resistances in wild pepper types is crucial to amplify genetic diversity [142]. Currently, wild species of pepper from gene banks have been screened and phenotypically characterized as being tolerant to salinity and water stress under control conditions, and then used as rootstocks in the field, where abiotic stress problems occur, and productivity of grafted plants has been evaluated [132, 143, 144].

*4.1.1. Grafting to cope with salt stress*

One of the several approaches followed to cushion the impact of salinity is to graft plants onto tolerant rootstocks [10], and is a common agronomic practice in melon and tomato. Some works into these species have been conducted to elucidate the mechanisms that are involved in grafted plants' increased salinity tolerance. Such increased tolerance is generally associated with plants' capacity to retain or exclude, and/or accumulate toxic ions, $Na^+$ and $Cl^-$ in rootstock roots. Hence, this action limits their transport to leaves instead of through the induction of antioxidant systems to the synthesis of osmotically active metabolites [35, 145]. Other authors have suggested that the rootstock's influence on the salt tolerance of scions is owing to stomatal functions (changes in stomatal regulation and water relations) being more efficient controlled. What this suggests is that grafting incisions could alter the hormonal signaling between shoots and roots [146]. In other cases, the re-establishment of ionic homeostasis has explained increased tolerance [124]. Yet in grafted plants, the mechanism of resistance against salinity displays a high degree of complexity in relation to specific scion/rootstock interactions [145, 147], and may vary among species. As far as we are aware, very few studies have been conducted into pepper to elucidate whether the salt tolerance conferred by rootstocks is due, or not, to retention and/or exclusion mechanisms, as in melon or tomato, because of them being better able to alleviate the toxic effects of salts or of other processes; e.g., water relations being maintained or antioxidant capacity being enhanced.

Salt tolerance among pepper genotypes may vary [72]. Maas [9] has indicated a salinity resistance threshold of 1.5 dS $m^{-1}$, and below which they found no effect on growth, but a 14% drop in biomass production for each additional 1 dS $m^{-1}$. Thresholds within the 0–2 dS $m^{-1}$ range and slopes of salinity response curves that go from 8 to 15% have been reported for greenhouse peppers [10]. Another example is to use irrigation water of 4.4 dS $m^{-1}$ [67], which resulted in reductions of 46% in the pepper dry biomass and of 25% in marketable pepper fruits. Guifrida et al. [109] have reported that stunted growth caused by salinity attenuates in pepper-grafted plants, compared with the non-grafted plants, is primarily associated with a low salt ions uptake. Therefore, these ions are present in the grafted plants at lower concentrations rather than leaf turgor being maintained by osmotic adjustments.

Different tolerance mechanisms to salt stress (NaCl 40–80 mM) were observed in our experiments using tolerant accessions (previously selected) like rootstocks and commercial "Adige" cultivar as a sensitive scion. Increased fruit yield under salinity when grafted onto accessions *Capsicum chinense* Jacq. "ECU-973" (code 12) and *Capsicum baccatum* L. var. pendulum "BOL-58" (code 14) was measured. Higher productivity under field conditions for these grafted plants was due to their ability to restrict $Cl^-$ transport to leaves, and also to reduced $Na^+$ loading in leaves and roots, which thus favored $K^+$ ($Ca^{2+}$ and $Mg^{2+}$) uptake and allowed a lower osmotic potential at less energy costs.

Such traits had a weak but negative impact on photosynthesis, nitrate reductase activity, and lipid peroxidation in the grafted scion leaves compared with ungrafted plants (Adige). Tolerance to salinity in these grafted plants was expressed to maintain scions' ion homeostasis, and can consequently improve crop yields [148, 149].

Nevertheless, by using *C. annuum* (code A25) as a tolerant rootstock, we also observed a larger amount of marketable fruit (+75%) and lower Blossom-end Root incidence (−31%) in commercial pepper cultivar Adige grafted onto A25 (A/A25) compared with ungrafted plants (**Figure 5A** and **B**), but the accumulation of $Na^+$ and $Cl^-$ in leaves and roots was similar in grafted or ungrafted plants. Another tolerant salt mechanism was found in this grafted plant. Despite continued salt ions uptake, A/A25 plants' buffer capacity was not superseded as a testimony by unaffected biomass production and photosynthesis. The high $Na^+$ and $Cl^-$, accumulations and their likely compartmentalization in the apoplastic space and/or vacuole to preserve the cytosol from ionic toxic effects could well occur. Tolerance may be attributed to the ability to maintain shoot/root growth under salt stress, which has been related to A/A25 plants' ability to limit, or protect, loss of $CO_2$ assimilation (**Figure 6**) and sink activity in growing organs [149, 150].

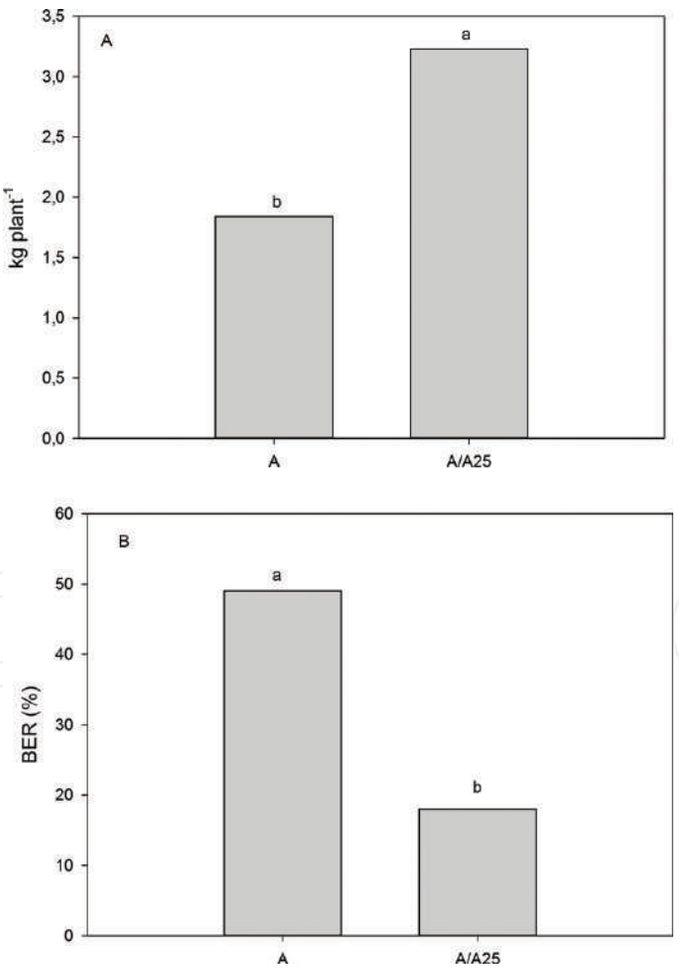


**Figure 5.** Marketable fruit yields (A) and the percentage of fruits affected by BER (B) under soil salinity and water conditions. Values are the mean of 50 replicates per cultivar Adige either ungrafted (A) or grafted onto the A25 genotype (A/A25). The different letters in each column denote significant differences at $P < 0.05$ according to the LSD test, and following a one-way ANOVA test by taking plant type as the variability factor.

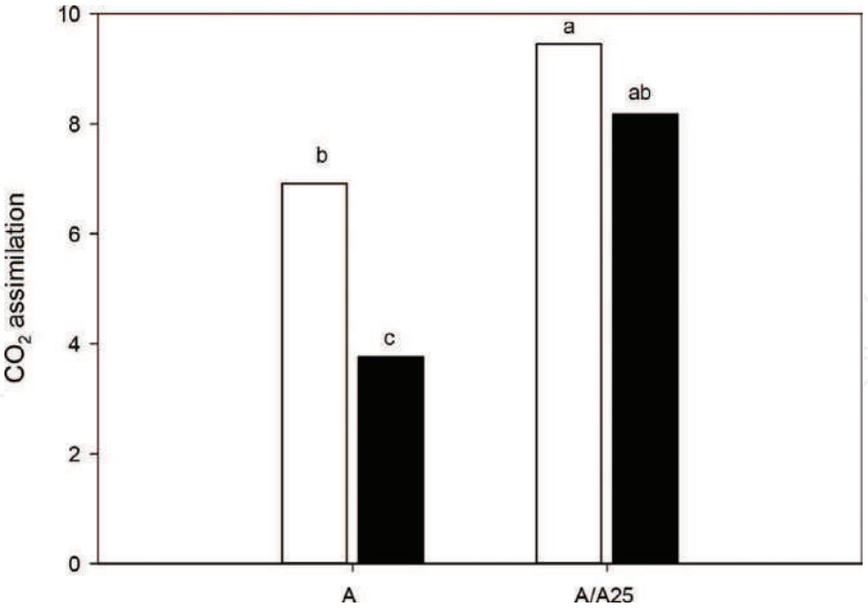


**Figure 6.** $CO_2$ assimilation (μmol $CO_2$ $m^{-1}$ $s^{-1}$) of the cultivar Adige ungrafted (A) or grafted onto the A25 genotype (A/A25) under control (white bars) and salinity conditions (black bars). The values are the means of four replicates per genotype. The different letters in each column denote significant differences at $P < 0.05$ according to the LSD test, and following a two-way ANOVA test with plant type and NaCl treatment taken as the variability factor.

To conclude, grafting commercial varieties onto salt-tolerant rootstocks can be considered a valid strategy for ameliorating salt tolerance in peppers.

*4.1.2. Grafting to overcome water stress*

A novel perspective to enhance resistance to water stress is to use tolerant accessions as rootstocks for a given and desirable commercial cultivar. The interactions that take place among the graft, water stress, and vegetable plants have been studied mostly in cucumber, melon [151], and tomato [130, 152] by centering on the growth effects of grafting, and also on its physiological effects, and particularly on photosynthesis traits and hydric relationships [153]. Grafted plants usually show increased uptake of water and minerals compared to self-rooted plants as a result of the vigorous root system used as the rootstock [130, 154, 155]. Greater SOD and CAT activities, higher proline accumulation levels, and lower lipid peroxidation levels have been found in tobacco scions grafted onto drought-tolerant rootstocks [156]. Tomato grafted onto a drought-tolerant line has shown not only reduced growth, but also water conservation, as well as increased photosynthetic rates under mild drought conditions [152]. Similar results have been obtained by Liu et al. [157] using luffa as rootstocks when grafted with either its scion or cucumber.

However, reports on the physiological alterations of pepper after grafting and exposure to water stress are limited. Deep pepper root systems have been considered one of most important traits of tolerance. López-Marín et al. [158] have reported finding greater root growth in drought-tolerant grafted pepper plants (Hermino grafted onto Atlante) compared with scions (Herminio) ungrafted in an irrigation-deficit regime. The physiological tolerant mechanisms to overcome water stress in pepper-grafted plants are not well-known. The

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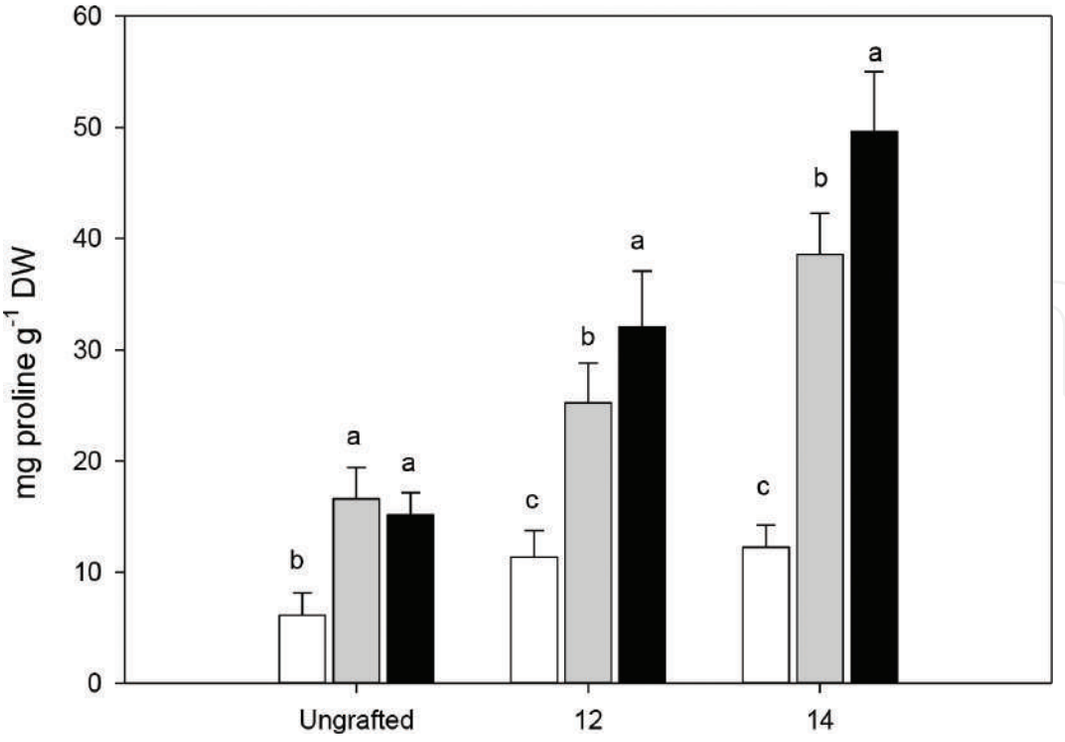


**Figure 7.** Changes in proline concentrations in leaves (mg proline $g^{-1}$ DW) from the ungrafted pepper plants (cultivar "Verset") and the cultivar grafted onto accessions 12 and 14 after adding PEG at 0% (white bars), 3.5% (gray bars) and 7% (black bars) during a 14-day exposure period. Data are the mean values ± SE for n = 6. Within each plant combination, different letters indicate significant differences at $P < 0.05$ (LSD test).

effect of adding 3.5% and 7% PEG (polyethylene glycol) was examined for 14 days in two drought-tolerant rootstocks (codes 12 and 14, see Section 4.1.1) to identify the physiological traits responsible for the tolerance provided by rootstocks compared with ungrafted plants [159]. In grafted plants, we observed a higher proline level (**Figure 7**), along with a significant decrease in the osmotic potential, which reflected the lesser reduction in RWC. Enhanced osmotic adjustment may protect leaves from excessive dehydration. However, our results indicated that the water stress effect depended on the duration and intensity of the stress level, and also on the rootstock used.

Considering the overall results published about grafts, grafted plants can act as an efficient tool to mitigate abiotic stress in the climate change context and a tolerant rootstock that can make water and salt stress vanish on scions to reach greater productivity and fruit quality [149]. Nonetheless, the physiological and genetic mechanisms for abiotic tolerance in grafted plants, especially in peppers, are still unknown.

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## Author details

Consuelo Penella and Angeles Calatayud*

*Address all correspondence to: calatayud_ang@gva.es

Departamento de Horticultural, Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, Valencia, Spain

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